What is known, new and controversial about GLP-1? Minutes of the 1st European GLP-1 Club Meeting, Marseille, 28–29 May 2008

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Abstract

The first antidiabetic agent was a hormone—insulin—and ever since, all therapeutic strategies have been based on the synthesis of chemical compounds to bind its receptors or transcription factors, or to trigger its intracellular mechanisms. Eighty years on, new therapeutic molecules are available for the treatment of diabetes and, again, are based on a hormone—glucagon-like peptide-1 (GLP-1). Whereas the theoretical benefit of insulin is based on normalization of functional physiology, therapeutic strategies based on GLP-1 aim to increase the circulating concentration of a natural component—the hormone GLP-1. There are two strategies for increasing GLP-1 plasma concentrations: replace the hormone with a long-acting analogue or molecule with a longer half-life; and prevent its degradation by inhibiting its natural protease, dipeptidyl peptidase IV (DPP-IV). Although numerous clinical studies have been carried out and vast amounts of data are available, the mechanisms through which GLP-1-based therapy reduces blood glucose in diabetic patients remain unclear. Thus, it is essential to ask the right questions and to design appropriate clinical trials and experiments to increase our understanding of the mode of action of GLP-1-based therapy. For this reason, in the spring of 2008, expert scientists and clinicians in the field of GLP-1 got together for an intensive debate on the subject at the first meeting of the European Club for the study of GLP-1, held in Marseille. The subject of the round table discussions was: what is known, new and controversial about GLP-1? During these discussions, numerous facts and controversies were reevaluated, and revealed that several long-held, dogmatic beliefs have never been fully and scientifically established. These points are detailed here in these minutes of the landmark meeting.

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Résumé


Le premier traitement du diabète était une hormone : l’insuline. Depuis, tous les antidiabétiques étaient dérivés de composés chimiques dirigés contre des récepteurs, des facteurs de transcription ou d’autres mécanismes intracellulaires. Quatre-vingt années plus tard, diverses molécules sont disponibles en thérapeutique et sont fondées une nouvelle fois sur une hormone intestinale, le GLP-1. Comme pour l’insuline, l’avantage conceptuel est fondé sur la normalisation des fonctions physiologiques contrôlées par cette hormone. Pour augmenter la concentration plasmatique de GLP-1, il existe deux stratégies. Dans la première, une forme stable de l’hormone est utilisée par voie injectable. Pour la seconde, un produit pharmacologique inhibe l’enzyme de dégradation du GLP-1, la dipeptidyl peptidase IV (DPP-IV). Bien que de nombreux essais cliniques aient été réalisés et de nombreuses données expérimentales soient disponibles, les mécanismes qui permettent à ces deux stratégies de corriger le diabète ne sont pas intimement démontrés. Ainsi, il est clairement nécessaire de définir les questions précises qui concernent les domaines d’incompréhension, de proposer des essais cliniques adéquats et des protocoles expérimentaux qui ciblent les mécanismes associés aux effets hypoglycémiants des molécules fondées sur le concept du GLP-1. Ainsi, au printemps 2008, des scientifiques et cliniciens experts dans le domaine du GLP-1 se sont rencontrés pour débattre intensément de ces questions et proposer de nouvelles approches. Ces premières rencontres du Club européen pour l’étude du GLP-1 se sont tenues à Marseille. Des tables rondes ont été organisées autour d’une question : que sait-on ? Que doit-on savoir ? Quels sont les éléments de controverse ? Puis, des questions précises ont été discutées et...
1. Roundtable topics

Among the first topics discussed, was the role of GLP-1 in the regulation of insulin secretion, which was addressed by Guy Rutter (Imperial College, London, UK), focusing on the well-known cAMP-dependent pathway. A key point of the discussion was the additive role of GLP-1 in the increase in intramitochondrial calcium associated with higher mitochondrial ATP content. The mimicry of forskolin, carbachol and the separate effects of K-ATP channel opening and GLP-1 were discussed in terms of their dominant role in glucose-induced insulin secretion. The additive and synergistic effects of calcium and the cAMP pathways were also noted, and led to the clear conclusion that efforts need to be directed towards understanding the importance of each pathway in diabetes. The difficult question of molecular targets in diabetes in terms of lipotoxicity and glucotoxicity was also addressed. The maintenance of functional GLP-1 signaling suggests that this peptide can overcome impaired molecular mechanisms. However, more experiments are needed to arrive at any definitive conclusions (Fig. 1).

In the second round of discussions, Jens Holst (Pannum Institute, Copenhagen, DK) addressed the key issues of measurements, suitability of cell lines, role of neuroendocrine regulation, and presence of specific regulators and secretion in pathological states. He first established that GLP-1 assays need to be interpreted with caution. In humans, most—if not all—of the active GLP-1 is amidated, which means that an antibody against the amidated form could reflect total plasma GLP-1 concentration. In animal models, a considerable amount of non-amidated GLP-1 is present and is not recognized by the primary antibody used in human assays. This may lead to inaccurate data from animal studies that the scientific community may not be aware of.

Holst then moved on to the regulation of GLP-1 and presented the cells lines available, none of which are perfect or well understood, and some of which are as yet incompletely characterized. This raises the question of the definition of an L cell, which has to be polarized—yet none of the current cell lines are. This was followed by a discussion of the distribution and heterogeneity of L cells along the gastrointestinal tract, as these cells co-express different peptides such as GIP, GLP-1, CCK and PYY, each with its own actions. It now seems clear that the L-cell gradient between the proximal and distal parts of the intestine is associated with considerable heterogeneity and probably with different regulators of secretion. In addition, it may be that the L cell is always in a dynamic state throughout its brief lifetime, with variable expression of different gut peptides. Such heterogeneity could be considered functional. Whether or not diabetes affects the secretory capacity or the heterogeneity of one group of L cells or another, and whether or not the co-expressed peptides are essential for the regulation of GLP-1 secretion was widely debated and certainly requires more data.

As the isolated intestine appears to be an ideal model for the study of GLP-1 secretion—it has a luminal side, and the cells are polarized, irrigated and innervated—more research should be directed towards this system.

In the third round of discussions, Rémy Burcelin (Institute of Molecular Medicine, Toulouse, France) focused on the role of brain GLP-1 signaling in the control of glucose homeostasis (not involving satiety). Given the results from his laboratory and in the literature, he discussed the regulatory role of brain GLP-1 signaling on insulin secretion, insulin resistance, hepatic glucose production and the increased number of brainstem GLP-1-expressing cells in obese rats. The key question remains the respective roles of brain GLP-1 signaling and enteric GLP-1 in the general physiological effects of GLP-1. In fact, this topic was repeatedly addressed by the other panel discussions and appears to be an important, unresolved question in the physiology of GLP-1. Clearly, scientific efforts should be directed towards dissecting and answering this...
question, which is now even more important because of the recent availability of drugs based on GLP-1 physiology such as DPPIV inhibitors, and GLP-1 analogues and receptor agonists. It was concluded that there is no definitive evidence of the importance of the direct enteric–pancreatic axis over the indirect enteric–brain–pancreatic axis. GLP-1-receptor knock-out mice are considered to be useful tools to help answer this key physiological question.

Glucagon action, secretion and the regulatory role of GLP-1 was then addressed by Jean Girard (Cochin Institute, Paris, France), who began by noting that diabetic patients have elevated plasma glucagon concentrations throughout the day that neither meals nor changes in glycaemia can regulate. This means that oral glucose intolerance is the result of non-suppression of glucagon secretion. The debate was then directed towards whether or not impaired GLP-1 secretion could explain such observations. It was proposed that the activation of glucose-sensitive sensors by GLP-1 should favour glucose-regulated glucagon secretion, and that the glucagon-lowering effect of GLP-1 is most likely an important regulator of glycaemic levels in diabetic patients. However, as yet, no clear answers have been found, and there are not enough reliable data to definitively demonstrate that the antidiabetic effects of GLP-1-based therapies are related to improved glucose-sensitive glucagon secretion. Again, more research is needed on this topic, which should be given priority status in the understanding of GLP-1 physiology and its application as an antidiabetic agent.

Steve Bloom (Imperial College, London, UK) addressed the question of the regulatory role of GLP-1 on body weight, energy expenditure and gastric-emptying. It is known that GLP-1 reverses weight gain, one of the peptide’s main physiological effects. Clinical trials have described the weight-loss effect of long-term treatment with GLP-1 analogues and receptor antagonist in type 2 diabetics, and the debate focused on determining whether or not the physiological effects can be attributed to satiety, or to a combination of energy expenditure and gastric-emptying. However, not enough data are available to answer this important question with certainty.

What is also still unclear is the importance and contribution of the vagus nerve and enteric nervous system versus enteric GLP-1. The trophic effects of GLP-1 on beta cells were addressed by Bernard Thorens (Department of Physiology and Center for Integrative Genomics, University of Lausanne, Switzerland). These effects were summarized as involving:

- differentiation from precursor cells and the preservation of fully differentiated phenotype ability to remain glucose-competent;
- proliferation of mature beta cells;
- and protection against apoptosis induced by inflammation and/or gluco- or lipotoxicity.

With the advent of DPPIV inhibitors, Carolyn Deacon (University of Copenhagen, DK) pointed out that plasma DPPIV activity is a minor contributor to degradation of GLP-1, and that the membranous form of DPPIV in tissues—in particular, vascular endothelium—is probably the most important source of the enzyme responsible for GLP-1 cleavage. Although DPPIV is present in the capillaries surrounding L cells, none is found in the interstitial fluid, suggesting that large amounts of GLP-1 could reach the myenteric plexus and activate the vagus nerve. This provocative thought could mean that DPP IV has a regulatory role in preventing overt, active plasma concentrations of GLP-1. The remaining intact GLP-1 might still trigger the portal vein sensor, but very little intact GLP-1 reaches the pancreatic beta cells to regulate insulin secretion via an endocrine mechanism.

Indeed, the question of the role of DPPIV on GLP-1 physiology still requires considerable research, given the paradoxical short half-life of the peptide and its distance from beta-cell receptor sites. This led to a discussion of the physiological role of the degradation product of GLP-1: GLP-1₉₋₃₆. Although controversial, there is evidence of a small effect on glycaemia independent of changes in insulin. Indeed, this byproduct accumulates rapidly in the circulation after a meal and, although its function remains to be fully assessed, a cardiovascular effect has been proposed. A putative second GLP-1 receptor was also discussed and, although no molecular evidence has yet been presented, there are circumstantial data to suggest that such receptors do exist. It was also noted that, in addition to GLP-1₉₋₃₆, a dipeptide is generated that could play a part that is, as yet, unknown. Again, research efforts should be engaged in this direction as well. The question of whether or not there are other physiologically relevant substrates was then raised, and ended with a consensus that DPP IV is a protease that in vitro may have numerous substrates as long as the buffers and experimental conditions are appropriate. GIP is certainly such a substrate, but the role of its physiological inactivation by DPPIV has yet to be elucidated.

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In pathological and physiological conditions of increased beta-cell mass such as obesity, neogenesis, replication of mature beta cells and reduction of beta-cell apoptosis could be under the control of GLP-1. These mechanisms have still not been identified and represent milestones for scientist to achieve. This was followed by further debate as to which experimental procedures, in vivo or in vitro, would be suitable for identifying the mechanisms responsible for these GLP-1 effects. Indeed, identi-
The duality of the cAMP–CREB pathway for the regulation of gene expression versus the cAMP–calcium activated pathway was also discussed to address which beta-cell pathways are altered in diabetes and overtly activated by GLP-1 receptor agonists and analogues. In rodents treated with GLP-1 receptor agonists, in pancreatectomized rats and in streptozotocin-treated newborn rats, beta-cell differentiation is thought to occur. However, a clear description of the differentiation process is needed and represents yet another area of further, important investigation.

It was concluded that the discovery of the mechanisms through which GLP-1 induces beta-cell differentiation would be helpful in determining future therapeutic strategies for both type 1 and type 2 diabetes. Another important question is whether differentiated cells are defined by expression of insulin or in association with ducts, and whether isolated cells could be considered part of the differentiation process. Similarly, was the effect of GLP-1 on precursor cells direct or indirect? A clear answer is not likely to be forthcoming, and there is speculation as to whether or not the differentiating effect of GLP-1 can be observed acutely or only with chronically repeated treatment. The discussion also considered the anti-apoptotic role of GLP-1, and its contribution to the therapeutic effect of GLP-1-based therapies. The difficulty most likely lies in assessing in humans, this mimicry was probably due to improved glucose control.

In his second set of slides, Gallwitz presented the dark side of such analogues and inhibitors. The nausea and antibody production were related to the effects of gastric-emptying, a point that was discussed at length, which ended with ascribing a minor role to nausea in the overall control of blood glucose concentrations and weight loss. This left the questions of whether or not the HbA1c reductions observed so far were sustained, and what sort of clinical studies are now required. The most recent data from long-term clinical trials show sustained effects, so the discussion shifted to possible side-effects such as pancreatitis. Although this issue needs to be explored, it was considered to have a minimum impact on health. Finally, the limitations of injectable therapies in clinical practice were raised. However, given the recent improvements in longer-lasting injected forms, this is no longer an issue.

At this roundtable meeting, many basic and clinical questions were raised and discussed at length, and led to the conclusion that the improved blood-glucose profiles obtained with the use of analogues and inhibitors was due to a combined action of insulin- and non-insulin-dependent effects. Although this idea had already been previously suggested, the dogma that incretin improves glycaemic profiles strictly through insulin secretion appears to be not true. This was concluded on the basis of discussions among experts who drew upon solid personal experience and on comparisons in the published data. The long-term future prospects were then discussed and are currently under construction.

The next meeting of the EuCSGLP-1 will be held in June 2009, with the objective of presenting new arguments and answers to some of the key questions raised here.

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